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Experimental **Hematology**

Rapid Communication

Recombinant Human Stem Cell Factor Synergises with GM-CSF, G-CSF, IL-3 and Epo to Stimulate Human Progenitor Cells of the Myeloid and Erythroid Lineages

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Abstract. The cDNA for human stem cell factor (hSCF) has been cloned and expressed in mammalian and bacterial hosts and recombinant protein purified. We have examined the stimulatory effect of recombinant human SCF (rhSCF) on human bone marrow cells alone and in combination with recombinant human colony stimulating factors (CSFs) and erythropoietin (rhEpo). RhSCF alone resulted in no significant colony formation, however, in the presence of rhGM-CSF, rhG-CSF or rhIL-3, rhSCF stimulated a synergistic increase in colony numbers. In addition, increased colony size was stimulated by all combinations. The morphology of cells in the colonies obtained with the CSFs plus rhSCF was identical to the morphology obtained with rhGM-CSF, rhG-CSF or rhIL-3 alone. RhEpo also synergised with rhSCF to stimulate the formation of large compact hemoglobinized colonies which stained positive for spectrin and transferrin receptor and had a morphological appearance consistent with normoblasts. RhSCF stimulation of low density non-adherent, antibody depleted, CD34+ cells suggests that rhSCF directly stimulates progenitor cells capable of myeloid and erythroid differentiation.

Key words: Stem cells - Stem Cell Factor - Synergy - Hemopoiesis

Recently a novel growth factor termed stem cell factor (SCF), was identified by its ability to stimulate primitive mouse progenitor cells [1]. In combination with interleukin 6 (IL-6) or macrophage colony stimulating factor (CSF-1), rat derived stem cell factor (rSCF) stimulated colony formation of high proliferative potential colony forming cells (HPP-CFC) [1,2]). The HPP-CFC have properties which correlate closely with the properties of mouse bone marrow cells with marrow repopulating ability [3]. rSCF has been purified to homogeneity from medium conditioned by Buffalo rat liver (BRL-3A) cells and partial amino acid sequence determined [4]. The cDNA for rSCF was cloned from BRL-3A cells and subsequently the human cDNA was isolated from HT-1080 and 5637 cells [4,5].

The properties of the recombinant rSCF (rrSCF) suggest that it is a multipotent factor acting on cells of the myeloid, mast and lymphoid lineages [1,4,6,7]. In semi solid agar culture, rrSCF stimulates mixed colony formation of neutrophil, macrophage and megakaryocyte cells [1,4]. The stimulation of mixed colony formation may be due to indirect effects of accessory cells rather than a direct effect of rrSCF. IL-7 synergises with rrSCF to stimulate pre-B cell colony formation in semi solid agar culture of normal mouse bone marrow cells [7]. Stem cell factor has been identified as the ligand for the tyrosine kinase type receptor, c-*kit* [[8]. In addition, stem cell factor has been shown to be the product of the *Sl* locus of the mouse, which is known to be essential for *in vitro* and *in vivo* stem cell survival [8-11]. These properties demonstrate that stem cell factor is a multilineage factor which may act directly upon a common lymphoid-myeloid stem cell.

In this study we have examined the ability of recombinant human SCF (rhSCF) to stimulate human progenitor cells and present data clearly showing synergistic interactions with recombinant human colony stimulating factors (CSFs) and recombinant human erythropoietin (rhEpo).

Materials and methods

Human bone marrow cells. Bone marrow cells were provided by Dr. P. Koeffler (UCLA Medical Center, Los Angeles, CA) from healthy normal adult volunteers who gave informed consent under UCLA Institutional guidelines. The cells were centrifuged over ficoll hypaque and the low density cells collected and washed with Hank's balanced salt solution supplemented with 2% BSA (HBSS). The low density cells were incubated overnight in culture dishes in HBSS and the non adherent fraction collected.

Agar culture of human bone marrow cells. Double layer agar cultures in 35 mm dishes were used as previously described [12]. Alpha modification of

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Eagle's MEM (Flow Laboratories, McLean, VA) supplemented with 20% FCS was used for all cultures. The number of cells plated is presented in the Results Section. Growth factors were incorporated in the underlays at a maximum of 13.2% of the total culture volume (1.5 ml) per dish. Cultures were gassed with a 5% O_2, 10% CO_2, 85% N_2 mixture and incubated at 37°C for 14 days after which time colonies were scored using a dissecting microscope. Colonies were scored as all colonies containing 50 or more cells.

Cell enrichment procedures. Low density non adherent cells were prepared as described above and pelleted after washing in PBS plus 2% BSA. The cells were then incubated for 60 min with a panel of monoclonal antibodies to mature cells as described previously [13]. Unlabelled antibody was removed by washing and the cells incubated with magnetic beads (Dynal Inc., Great Neck, NY), coated with goat anti mouse IgG, for 60 minutes with mixing at a ratio of 1:5 cells to beads in 6 ml final volume. The positive cells were removed using a magnetic particle separator (Dynal Inc., Great Neck, NY) and the antibody negative cells used in agar cultures as described above. Cells bearing CD34 antigen were isolated from the antibody negative cells by direct immune "panning" as previously described [14].

Growth factors. Recombinant growth factors were prepared as previously described; human granulocyte-macrophage CSF (rhGM-CSF) [15], human granulocyte CSF (rhG-CSF) [16] and human interleukin 3(rhIL-3) [17] were purified from inclusion bodies formed in *E. coli* containing the appropriate expression plasmid. Recombinant human erythropoietin (rhEpo) [18] was purified from medium conditioned by chinese hamster ovary containing the appropriate expression plasmid. Recombinant human SCF^{162} was expressed in COS-1 cells and recombinant human SCF^{164} was purified to greater than 99% purity from *E. coli* [5]. Plateau doses of each factor were determined from dose response curves and used for culture. These doses were: rhGM-CSF - 62.5 ng (10^7 U/mg), rhG-CSF - 100 ng (10^8 U/mg), rhIL3 - 70 ng (10^7 U/mg), rhEpo - 4 units, and 100 ul of $rhSCF^{162}$ from COS-1 cell supernatant (diluted 1:2) and 124 ng of purified $rhSCF^{164}$ (*E. coli* derived) per culture dish.

Results

The gene encoding human SCF [amino acids 1-162 ($rhSCF^{162}$)] was inserted into a mammalian expression vector, v19.8, and transfected into COS-1 cells [5]. $RhSCF^{162}$ alone stimulated no colony formation in agar cultures of normal human bone marrow, however, a synergistic increase in colony numbers resulted with the combination of $rhSCF^{162}$ plus rhIL-3, rhG-CSF, rhGM-CSF or rhEpo (Table 1). Addition of $rhSCF^{162}$ to rhGM-CSF, rhG-CSF and rhIL-3 resulted in 7, 4.5 and 1.8 fold higher colony numbers respectively, compared to the colony numbers obtained with the CSFs in the absence of $rhSCF^{162}$. The addition of $rhSCF^{162}$ had no effect on the morphology of the cells within the colonies formed. $RhSCF^{162}$ plus rhG-CSF stimulated colonies of neutrophils, $rhSCF^{162}$ plus rhGM-CSF stimulated colonies of neutrophils and macrophages, while $rhSCF^{162}$ plus rhIL-3 stimulated mixed colonies of neutrophils/macrophages and megakaryocytes. RhEpo alone stimulated no colonies, while addition of $rhSCF^{162}$ resulted in an average of 27 colonies per dish. Supernatants from COS-1 cells transfected with v19.8 (no insert) alone stimulated no colony formation and did not increase the numbers of colonies in the presence of any other factors (Table 1).

Table 1:
14 Day Colony formation in Cultures of Low Density Non Adherent Normal Bone Marrow Cells

Factors	Colonies[a] per 50,000 cells -rhSCF	+$rhSCF^{162}$[b]	mock COS-1[c]
Saline	0	0	0
rhGM-CSF	7.3 ± 1.2	50.7 ± 6.4	4.7 ± 0.5
rhG-CSF	10.3 ± 1.5	46.3 ± 1.4	10.3 ± 2.0
rhIL-3	9.3 ± 0.7	17.0 ± 3.1	7.3 ± 0.3
rhEpo	0	27.0 ± 3.1	1.0 ± 0

[a] Low density non adherent (LDNA) bone marrow cells were plated at 50,000 cells per dish in the presence or absence of $rhSCF^{162}$ from COS-1 cell supernatant and incubated for 14 days. The colony numbers presented are the means of triplicate dishes ± SEM.
[b] Supernatants from COS-1 cells transfected with v19.8:$hSCF^{162}$
[c] Supernatant from COS-1 cells transfected with v19.8 (no insert).

There was an increase in colony numbers stimulated by *E. coli* derived $rhSCF^{164}$ in conjunction with colony stimulating factors and rhEpo (Fig. 1). This synergy was observed at virtually all doses of CSFs or rhEpo used in the cultures. At 5ng or less, rhGM-CSF, rhG-CSF and rhIL-3 alone have very little effect on colony formation, however, with the addition of $rhSCF^{164}$ marked colony formation was obtained (Fig. 1). Similarly at low levels of rhEpo (0.25 U/culture or less) which have little stimulatory effect in combination with rhIL-3, $rhSCF^{164}$ plus rhEpo gave rise to significant colony formation (Fig. 1).

In addition to increased colony numbers in the presence of $rhSCF^{164}$, an increase in colony size was also observed for cultures containing $rhSCF^{164}$ plus rhGM-CSF, $rhSCF^{164}$ plus rhG-CSF and $rhSCF^{164}$ plus rhIL-3. An example of the increase in colony size obtained is presented in Figure 2 for $rhSCF^{164}$ plus rhGM-CSF compared to rhGM-CSF alone.

The combination of $rhSCF^{164}$ plus rhEpo resulted in the appearance of large hemoglobinized colonies (Fig. 3). The sizes of colonies stimulated by $rhSCF^{164}$ plus rhEpo reached up to 1.5 mm in diameter (Fig. 3). Cultures containing rhIL-3 plus rhEpo result in dispersed hemoglobinized colonies which contained much lower cell numbers per colony (data not presented). A comparison of the numbers

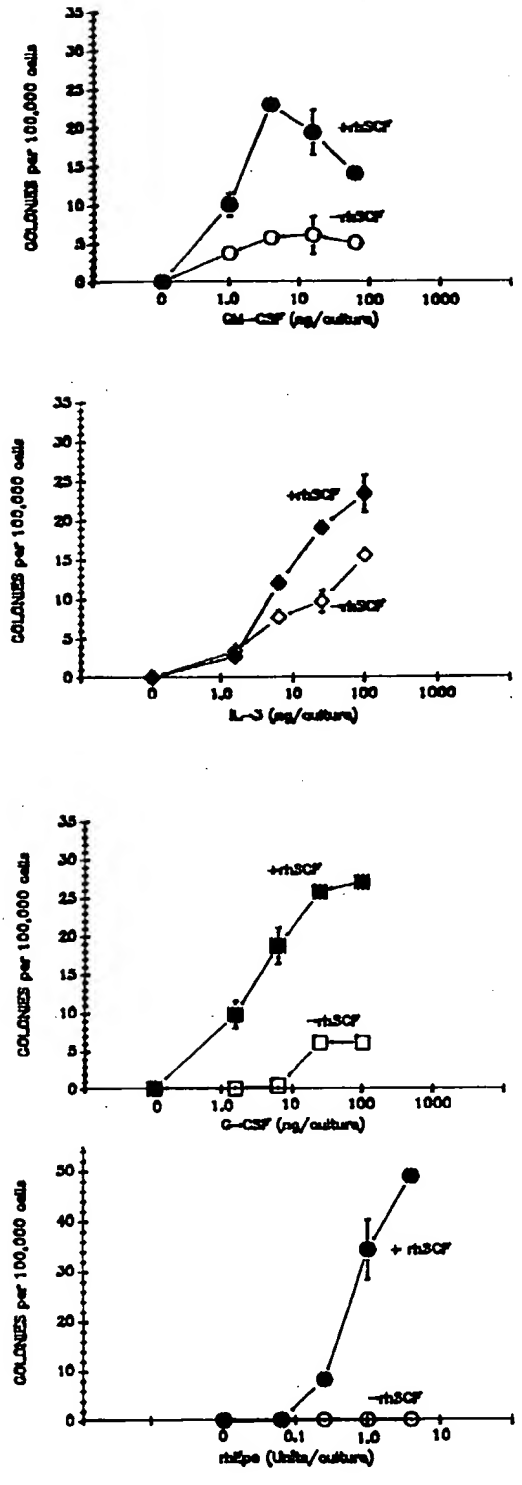

Fig. 1. Colony formation in agar culture of LDNA bone marrow cells in the absence or presence of rhSCF^{164}. A constant dose of rhSCF^{164} (124 ng in 100 ul) was plated with varying doses of the CSFs and rhEpo. Each point is the mean of triplicate dishes ± SEM.

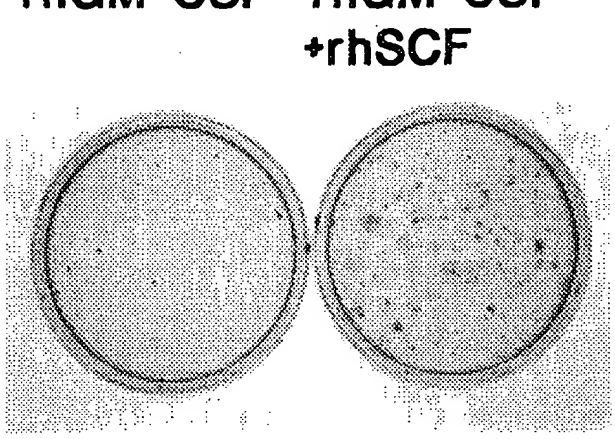

Fig. 2. Colony formation stimulated by rhGM-CSF alone or in combination with rhSCF^{164}. Cultures were stained with INT (1 mg/ml in HBSS; Sigma Chemicals, St. Louis, MO) and photographed. The plates have a diameter of 35mm.

of colonies stimulated by rhSCF^{164} plus rhEpo (36 colonies) and rhIL-3 plus rhEpo (27 colonies) to the three factor combination (58.5 colonies) shows an additive effect of rhSCF^{164} and rhIL-3 stimulated erythroid bursts. This observation is consistent with the interpretation that rhSCF and rhIL-3 stimulate a different progenitor cell population which is responsive to rhEpo. Cytospin preparations of the bursts stimulated by rhSCF^{164} plus rhEpo were stained with Wright-Giemsa. The morphology of the cells was primarily blast like, with some cells clearly identifiable as erythroid lineage. Most of the cells had a high nuclear to cytoplasmic ratio, prominent nucleoli, and blue staining cytoplasm indicative of their immature state (Fig. 4A). Colonies stimulated by rhSCF^{164} plus rhGM-CSF contained macrophage like cells and are shown for comparison (Fig. 4B). To examine the percentage of cells containing positive erythroid lineage markers from cultures containing rhSCF^{164} plus rhEpo, colonies were extracted from the agar cultures with a capillary pipet, washed in PBS containing 2% BSA, labelled with murine monoclonal antibodies to the transferrin receptor or spectrin and incubated with FITC conjugated anti mouse IgG. Positive fluorescence was observed in greater than 99% of cells for both transferrin receptor and spectrin. Myeloid cells from colonies formed in cultures of rhSCF^{164} plus rhGM-CSF were also stained with antibodies to the transferrin receptor and spectrin and no fluorescent labelling was observed.

The ability of rhSCF^{164} to stimulate colony formation by enriched progenitor populations was examined. Mature cells were removed using a panel of antibodies and magnetic separation. Early progenitors were further enriched by positive selection for cells expressing the CD34 antigen. These lineage negative CD34+ cells were plated at a density of 4,000 cells per dish with combinations of growth factors. Cultures of these

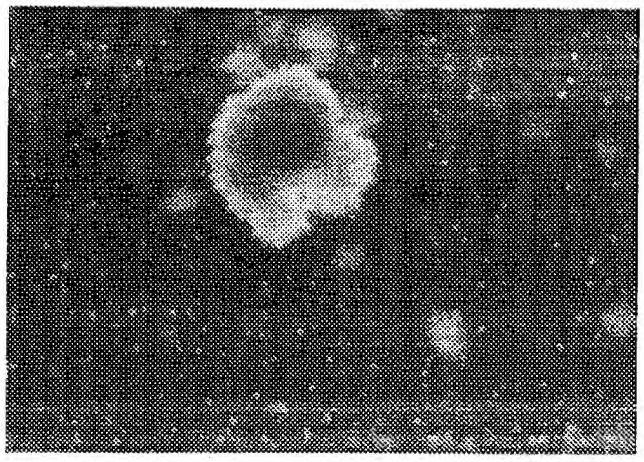

Fig. 3. Hemoglobinized colony from culture of rhSCF^{164} plus rhEpo. The colony was photographed in situ at a 25 X magnification.

cells resulted in similar synergistic increases in colony numbers with the addition of rhSCF^{164} (Table 2). RhSCF^{164} synergised with rhGM-CSF, rhG-CSF, rhIL-3 and rhEpo giving increased colony numbers and colony size, suggesting that the actions of rhSCF^{164} are direct and not mediated via accessory cells.

Table 2:
14 Day Colony formation in Cultures of Antibody Depleted and CD34+ Normal Bone Marrow Cells

Factors	Colonies (per 100,000 Cells) -rhSCF^{164}	+rhSCF^{164}
A: LDNA/Antibody depleted cells:		
Saline	0	0
rhGM-CSF	123.3 ± 4.0	340.0 ± 0.8
rhG-CSF	56.7 ± 0.3	196.7 ± 1.4
rhIL-3	63.3 ± 0.3	146.6 ± 1.9
rhEpo	0	163.3 ± 0.3
B: LDNA/Antibody depleted/CD34+ cells		
Saline	0	0
rhGM-CSF	541.7 ± 2.2	1025.0 ± 3.1
rhG-CSF	141.6 ± 0.5	416.7 ± 0.7
rhIL-3	275.0 ± 0.8	558.3 ± 2.4
rhEpo	0	175.0 ± 0.9

Low density non adherent (LDNA), antibody depleted bone marrow cells were plated at 10,000 cells per dish and CD34+ cells were plated at 4,000 cells per plate. The colony numbers presented are the means of triplicate dishes ± SEM and are expressed as the number of colonies per 100,000 cells.

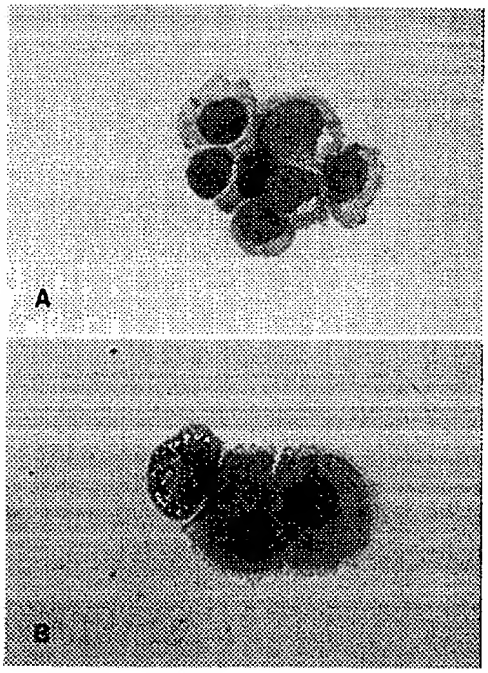

Fig. 4. Colonies from cultures containing rhSCF^{164} plus rhEpo (A) or rhSCF^{164} plus rhGM-CSF (B) were picked up, cytocentrifuge slides prepared and stained with Wright Giemsa stain. The cells were photographed at 100 X magnification.

In all combinations of rhSCF^{164} plus CSFs, enrichment of progenitors was obtained by positive selection for cells expressing the CD34 antigen compared to lineage negative cells (Table 2). The progenitors stimulated by rhSCF^{164} plus rhEpo were not enriched by positive selection for cells expressing the CD34 antigen suggesting that a subpopulation of BFU-e are CD34 negative and distinct to previously described BFU-e.

Discussion

In this study we have demonstrated a stimulatory role of COS-1 derived rhSCF^{162} and *E.coli* derived rhSCF^{164} on human bone marrow progenitor cells in combination with rhGM-CSF, rhG-CSF, rhIL-3 or rhEpo. RhSCF synergised with these factors giving increased colony numbers and colony size. The morphology of cells within the colonies stimulated with combinations of rhSCF was the same as that obtained with rhGM-CSF, rhG-CSF or rhIL-3 alone. Furthermore in the human system, rhSCF is able to stimulate multiple lineages in combination with the more lineage restricted CSFs. This suggests that in the human system, rhSCF stimulates early progenitors to become responsive to rhGM-CSF, rhG-CSF, rhIL-3 or rhEpo; or that rhSCF synergises with the CSFs and rhEpo to stimulate cells which require the presence of two factors for

proliferation and differentiation. In support of the second possibility, as determined by delayed addition experiments, both factors appear to be required from initiation of culture (data not shown). The increased colony numbers and colony sizes obtained with combinations of rhSCF and CSFs, suggest that cells with higher proliferative potentials are stimulated by these combinations of growth factors. This dual growth factor requiring population appears to represent a more primitive cell type than the population of cells responsive to single factors. This is consistent with the data obtained in the mouse system where recombinant rat SCF^{164} ($rrSCF^{164}$) has been shown to act on primitive progenitor cells of the myeloid and lymphoid lineages. $RrSCF^{164}$ alone stimulates the formation of colonies containing neutrophils, macrophages and megakaryocytes [1] and acts in combination with CSF-1, IL-6, GM-CSF and G-CSF to stimulate HPP-CFC [1; Zsebo and McNiece, unpublished data]. In combination with rhIL-7, $rrSCF^{164}$ stimulates early B cell progenitors, giving rise to colonies of pre-B cells in agar culture of normal mouse bone marrow cells [7]. In both the mouse and human studies, recombinant SCF stimulates colony formation in low density cultures where the populations have been depleted of mature cell types. This suggests the actions of SCF are direct and not mediated via accessory cell factor production.

The data presented in Figure 1 demonstrate the ability of rhSCF to potentiate the proliferative stimulus provided by other factors. This effect was observed at virtually all concentrations of the other factors examined. RhSCF can decrease the second factor threshold concentration necessary to stimulate colony formation by almost 1-2 logs. The levels of CSFs produced by stromal cells *in vivo* are much lower than the levels used in *in vitro* culture. SCF is produced by stromal cells of the bone marrow [7] and the decreased requirements for CSFs in the presence of SCF for stimulation of progenitor cells may represent a more physiological condition. With the advent of combination growth factor therapies for hematological abnormalities, SCF addition may prove useful in lowering the dosages for other growth factor such that harmful side effects are minimized without loss of effectiveness.

Stem cell factor may either act directly on a common myeloid/lymphoid stem cell or on distinct myeloid and lymphoid stem cells. Whether stem cell factor alone is able to induce proliferation of these stem cells or whether other factors are required still remains to be determined. Lineage commitment appears to be controlled by the presence of a factor such as GM-CSF or IL-7. These CSFs probably act in combination with stem cell factor to generate committed progenitors which can then respond to the lineage specific factors alone to induce proliferation and differentiation.

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References

1 Zsebo K, Martin F, Suggs S, Wypych J, Lu H, McNiece I, Medlock E, Morris F, Sachdev R, Tung W, Birkett N, Smith K, Yuschenkoff V, Mendiaz E, Jacobson F, Langley K (1990). Biological characterization of a unique early acting hematopoietic growth factor. Exp. Hematol. 18(6):703, (abstr.)

2 Bradley TR, Hodgson GS (1979). Detection of primitive macrophage progenitor cells in mouse bone marrow. Blood 54:1446

3 McNiece I, Bertoncello I, Kriegler A, Quesenberry P (1990). Colony forming cells with high proliferative potential. Int. J. Cell Cloning 8:146

4 Zsebo K, Wypych J, McNiece I, Lu H., Smith K, Karkare S, Sachdev R, Yuschenkoff V, Birkett N, Williams L, Satyagal V, Tung W, Bosselman B, Mendiaz E, Langley K (1990). Identification, purification, and biological characterization of hematopoietic stem cell factor from Buffalo Rat Liver conditioned medium. Cell 63:195

5 Martin F, Suggs S, Lu H, Langley K, Johnson M, Okino K, Morris C, McNiece I, Jacobsen F, Johnson M, Parker V, Flores J, Patel A, Birkett N, Smith K, Fisher E, Erjavec H, Herrera C, Mendiaz E, Wypych J, Sachdev R, Pope JA, Zsebo K (1990). Primary structure and functional expression of rat and human stem cell factor DNAs. Cell 63:203

6 Medlock E, Yung Y, McNiece I, Langley K, Mineo Daley C, Dubbell C, Mendiaz B, Sachdev R, Smith K, Zsebo K. Role of stem cell factor (SCF) in the stimulation of the mast cell lineage. Blood (In Press) (abstr.)

7 McNiece I, Langley K, Zsebo K. The role of stem cells factor in B cell differentiation: synergistic interaction with IL-7. Submitted J. Immunol.

8 Zsebo K, Williams D, Geissler E, Broudy V, Martin F, Atkins H, Hsu R-Y, Birkett N, Okino K, Murdock D, Jacobsen F, Langley K, Smith K, Takeishi T, Cattanach B, Galli S, Suggs S. Stem cell factor (SCF) is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 63:213

9 Dexter T, Moore M (1977). In vitro duplication and cure of hematopoietic defects in genetically anaemic mice. Nature 268:412

10 Ploemacher R, Molendijk W, Brons N, Ruiter H (1986). Defective support of Sl/Sl^d splenic stroma for humoral regulation of stem cell proliferation. Exp. Hematol. 14:9

11 Wright E, Lorimore S (1987). Haemopoietic stem cell proliferation in the bone marrow of Sl/Sl^d mice. Cell Tissue Kinet. 20:301

12 Bradley T, Hodgson G, Rosendaal M (1978). The effect of oxygen tension on haemopoietic and fibroblast cell proliferation in vitro. J. Cell. Physiol. 94:517

13 Emerson S, Sieff C, Wang E, Wong G, Clark S, Nathan D (1985). Purification of fetal hematopoietic progenitors and demonstration of recombinant multipotential colony-stimulating activity. J. Clin. Invest. 76:1286

14 Sealand S, Caux C, Favre C, Aubry J, Mannoni

P, Pebusque M, Gentilhomme O, Otsuka T, Yokota T, Arai N, Arai K, Banchereau J, de Vries J (1988). Effects of recombinant human interleukin-3 on CD34-enriched normal hematopoietic progenitors and on myeloblastic leukemic cells. Blood 72:1580

15 Boone T, Crandall L, Tsai K, Zsebo K (1987). Purification of recombinant human granulocyte-macrophage colony stimulating factor from E.coli. J. Cell. Biochem. 11c:176 (abstr.)

16 Souza L, Boone T, Gabrilove J, Lai P, Zsebo K, Murdock D, Cahzin V, Bruszewski J, Lu H, Chen K, Barendt J, Platzer E, Moore M, Mertelsmann R, Welte K (1986). Recombinant granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science 232:61

17 Yang Y-C, Ciarletta A, Temple P, Chung M, Kovacic S, Witek-Giannotti J, Leary A, Kriz R, Donahue RE, Wong GG, Clark SC (1986). Human IL-3 (multi CSF): identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. Cell 47:3

18 Lin F, Suggs S, Lin C, Browne J, Smalling R, Egrie J, Chen K, Fox G, Martin F, Stabinsky Z, Badrawi S, Lai PH, Goldwasser E (1985). Cloning and expression of the human erythropoietin gene. Proc. Natl. Acad. Sci. USA 82:7580